DTIC FILE COPY

DTIC
ELECTE
MAR 0 9 1988
E

Carbamate-Induced Performance and Thermoregulatory Decrements Restored with Diazepam and Atropine

CANDACE B. MATTHEW, M.S., ROGER W. HUBBARD, Ph.D.,
RALPH P. FRANCESCONI, Ph.D., and
GLENN J. THOMAS, B.S.

*U.S. Army Research Institute of Environmental Medicine,
Natick, Massachusetts*

AD-A192 667

MATTHEW CB, HUBBARD RW, FRANCESCONI RP, THOMAS GJ. *Carbamate-induced performance and thermoregulatory decrements restored with diazepam and atropine.* Aviat. Space Environ. Med. 1987; 58:1183–7.

When rats (500 g, male) are exercised on a treadmill, pretreatment with the carbamate physostigmine reduces endurance capacity (run time, RT) and increases the rate of rise of core temperature (heating rate, HR). Because physostigmine is a potential nerve agent pretreatment drug, our objective was to determine whether pharmacological intervention could reverse these decrements in performance and thermoregulation. The following drugs were administered separately via tail vein: vehicle-control (C), atropine (200 $\mu g \cdot kg^{-1}$, A), diazepam (500 $\mu g \cdot kg^{-1}$, D), and physostigmine (200 $\mu g \cdot kg^{-1}$, PH). After drug administration, rats were run (11 $m \cdot min^{-1}$, 6° elevation, Ta = 26°C) to exhaustion. PH administration resulted in reduced RT (41 min PH vs. 53 min C, p < 0.05) with greater HR (0.090°C·min^{-1} PH vs. 0.057°C·min^{-1} C, p < 0.01) than control rats. However, when A and D were also given to PH treated rats, the RT and HR were restored to control levels. Further, A and D without PH improved RT and HR (82 min, 0.047°C·min^{-1}) over control levels. Serial administration of an anticholinergic, an anticonvulsant, and an anticholinesterase resulted in no significant change in performance from control levels.

ANTICHOLINERGIC and anticholinesterase drugs, used therapeutically or prophylactically to protect against organophosphate poisoning in military settings, may have undesirable side effects (5). Thus, we have been primarily interested in the effects of these drugs on the physical, physiological, and thermoregulatory responses to heat and exercise, since physical performance and thermoregulation may be markedly effected by the prophylactic, therapeutic, or accidental use of these drugs.

Atropine, the prototype of anticholinergic drugs (5), in-

hibits evaporative cooling in man by suppressing sweat production (3) and in rats by suppressing saliva production which is behaviorally spread for evaporative cooling (6). Hubbard *et al.* (8) compared the effects of restraint, surgical desalivation, and chemical desalivation with atropine on the ability of rats to thermoregulate in the heat and reported that atropine inhibited thermoregulation to the same extent as surgical desalivation combined with physical restraint. We then used the heat-stressed rat (16) to determine the dose-response effects of atropine, and demonstrated that the rate of rise of core temperature (heating rate) of the rat was the most sensitive index of anticholinergic activity. Therefore, we used the relative heating rates of other anticholinergic drugs to determine a potency for these drugs relative to atropine, and also quantitated the relative ability of various carbamates to reverse the atropine-induced increase in heating rate as a measure of anticholinesterase potency (15). Salivation and sweating are under muscarinic cholinergic control. In the present study we have extended our model to include nicotinic effects of anticholinergic and anticholinesterase drugs in the exercising rat. Previous work from this laboratory has established the running rat as a model for human exercise-induced heat injury (9,10). Therefore, we are now examining both performance and thermoregulatory effects of these drugs in our rat model.

For many years the standard treatment for organophosphate anticholinesterase intoxication has been postexposure treatment with atropine and an oxime. Currently, however, there has been much interest in the use of the carbamate anticholinesterase pyridostigmine as a prophylaxis against nerve agents. Recent research has indicated that pretreatment with either carbamate physostigmine or pyridostigmine, in combination with atropine, significantly improved protection against the lethality of soman intoxication, and that physostigmine, but not pyridostigmine, afforded a mea-

88 3 03 013

sure of protection against soman-induced physical incapacitation (7). Physostigmine (PH) also is efficacious against anticholinergic syndrome (1). Thus, because of its potential efficacy as a prophylactic anticholinesterase, we wished to examine the effects of PH on thermoregulation and physical performance in an animal model. Atropine was chosen because of its role as a primary treatment drug and to counteract the muscarinic side effects of PH, while diazepam (D) was selected to limit the nicotinic side effects of PH (5). Diazepam has also been used with atropine in the treatment of organophosphate intoxication (24), and PH has been used to reverse the effects of excessive D in both man (14) and rats (20). Thus, our objective was to quantitate any performance or thermoregulatory decrement induced by the administration of PH or A in the running rat, and to attempt to restore this decrement by pharmacological intervention.

MATERIALS AND METHODS

Experimental Animals: Eight groups of ten adult male Sprague-Dawley rats (Charles River, CD strain, 510–530 g) were used one time only in all studies. The animals were caged individually in wire-bottomed cages and housed in an environmental chamber (4 × 3 × 2 m) maintained at 26°C and 50% rh. Lighting was controlled automatically (on, 0600–1800 h) and Purina rat chow and water were available *ad lib* except during experimental intervals.

Drugs: Prior to running, each rat received three separate injections 10 min apart via a lateral tail vein. The drugs, doses, and order of administration for each of the eight groups are presented in Table I. Atropine (A, 200 μg·kg^{-1}, as the sulfate, Sigma Chemical Co.) was dissolved in 0.2 ml of sterile 0.9% saline; diazepam (D, 500 μg·kg^{-1}, Valium*, Hoffmann-LaRoche Inc.) was diluted to 0.5 ml with fresh rat serum; and physostigmine (PH, 200 μg·kg^{-1}, Antilirium, Forest Pharmaceuticals) was diluted to 0.2 ml with saline. The order of drug administration (Table I) was selected (A first and PH last) because A was expected to have the longest duration of action while PH the shortest. Each drug dose used is within the human clinical range for the respective drug when the formula of Freireich *et al.* (4) is applied.

Experimental Procedure: The rats were weighed 15 min after the final (3rd) injection and then fitted with thermistors to measure core temperature (T_c, 6.5 cm insertion) and tail skin temperature (T_t, midlength, dorsum); then they were placed on the treadmill. The rats were run at 11 m·min^{-1} and 6° incline at an ambient of 26°C and 50% rh until they were exhausted (unable to right themselves when placed on their backs). At exhaustion the animals were removed from the treadmill and allowed to recover. During the run and recovery T_c and T_t were monitored and the shocker on the treadmill was controlled by a HP9825 computer-controlled data acquisition system (17).

Statistical Analysis: The data were analyzed by a one way analysis of variance followed by Tukey's test for all pair comparisons, or by Student's unpaired t test (Fig. 2). The null hypothesis was rejected at p < 0.05.

RESULTS

Fig. 1 illustrates that endurance, as measured by run time to exhaustion, is inversely correlated with heating rate (rate

TABLE I. DRUGS, DOSES, AND ORDER OF ADMINISTRATION.

DRUGS

C = vehicle control: 0.2 ml saline + 0.5 ml serum + 0.2 ml saline.
A = atropine: 200 μg·kg^{-1} in 0.2 ml saline.
D = diazepam: 500 μg·kg^{-1} in 0.5 ml serum.
PH = physostigmine salicylate: 200 μg·kg^{-1} in 0.2 ml saline.

Group	Injections*		
	1st	2nd	3rd
C	saline	serum	saline
A	A	serum	saline
D	saline	D	saline
A + D	A	D	saline
PH	saline	serum	PH
A + PH	A	serum	PH
D + PH	saline	D	PH
A + D + PH	A	D	PH

* 10 min apart, via lateral tail vein.

of increase in core temperature). The figure clearly demonstrates that the PH (cholinesterase = 60% of control) group had the shortest run time and the highest heating rate of the eight groups. This elevated heating rate cannot be attributed to the tremors observed in this group because at the start of run (15 min after the last injection), by which time the tremors were subsiding, T_c was lower than that of control rats (Table II). The A + PH group had a mean endurance time and heating rate that were not significantly different from those of controls: however, the A + PH group did exhibit tremors which were abolished by diazepam in the A + D + PH group. The combination of A + D significantly (p < 0.05) improved endurance over controls and all groups receiving PH. A useful measure of work done when comparing animals of different sizes is the kg·m [kg.m = body wt (kg) × run time (min) × speed (m·min^{-1}) × treadmill inclination (sin)]. As would be expected when all the animals are the same size and run at the same speed and inclination, the kg·m for the groups in Fig. 1 increase with endurance time. The mean (±S.E.) of the kg·m for some of the groups (Fig. 1) are: PH- 24 ± 3, C-31 ± 3, A + D + PH- 38 ± 2, and A + D- 49 ± 5; therefore, the A + D group did twice the work of the PH group, and significantly (p < 0.05) more than the control group.

Weight (wt) loss during the treadmill run (Table II) is the sum of wt lost through urination, defecation, and salivation (respiratory water loss is negligible). Total % water loss is also a function of the run time which ranged from 41 min for the PH group to 82 min for the A + D group (Fig. 1). Atropine decreases both fecal and salivary water loss (8) which explains the lower wt loss rate (Table II) in the A and A + D groups. Administration of the anticholinesterase PH, which stimulates both salivation and defecation (5), neutralized the A effect on wt loss in the A + PH and A + D + PH groups.

The restraint and multiple injection procedures raised the mean T_c of C rats from 37.5 ± 0.1°C preinjection to 38.7 ± 0.2°C at SOR (start of run, 15 min after the 3rd injection). There was a distinct division of the groups (Table II) into those with high SOR T_c (C, A, D, A + PH) and those with lower SOR T_c (A + D, PH, D + PH, A + D + PH). Except for the A group, whose mean T_c EOR (end of run) was

Fig. 1. Run time and heating rate for each of the drug treatment groups (see Table I for a key to the groups). Values are mean ± S.E.; * indicates a significant difference (p < 0.05) from the pH group.
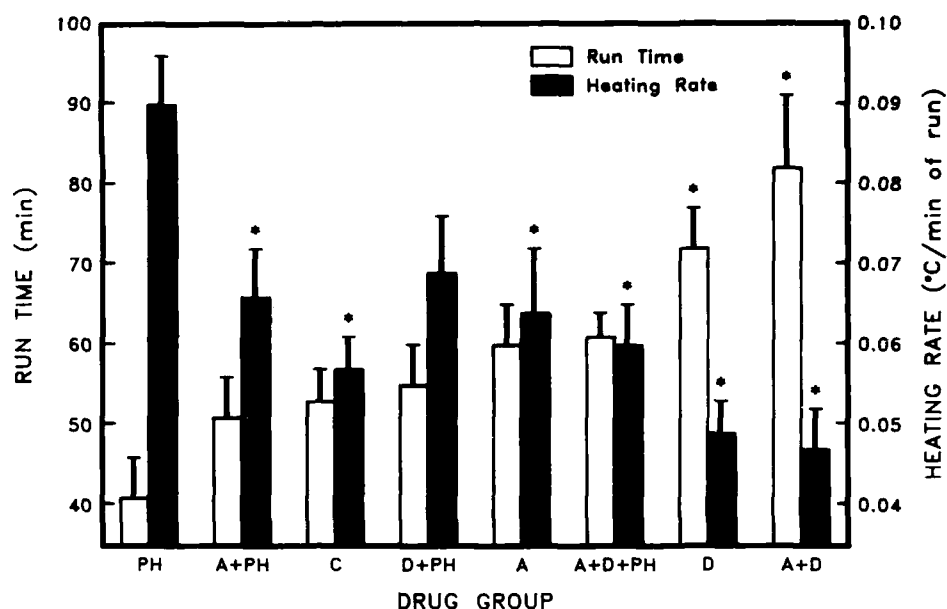
TABLE II. WEIGHT LOSS AND TEMPERATURE CHANGES IN THE RUNNING RAT.

Group (N = 10)	% Wt loss (%)	Wt loss/min run (g·min⁻¹)	Tc^a SOR (°C)	Tc^b EOR (°C)	Ti^c SOR (°C)
C	3.0 ± 0.2^d	0.31 ± 0.03	38.6 ± 0.2	41.6 ± 0.2	28.5 ± 0.5
A	1.5 ± 0.2*	0.13 ± 0.02*	38.7 ± 0.1	42.2 ± 0.2*	28.2 ± 0.5
D	4.0 ± 0.2	0.29 ± 0.01	38.5 ± 0.1	41.8 ± 0.1	29.5 ± 0.3
A + D	2.6 ± 0.2	0.18 ± 0.02*	38.1 ± 0.1*	41.5 ± 0.1	28.6 ± 0.5
PH	2.9 ± 0.2	0.38 ± 0.02	37.8 ± 0.1*	41.3 ± 0.1	31.8 ± 0.3*
A + PH	2.7 ± 0.3	0.28 ± 0.02	38.6 ± 0.2	41.8 ± 0.1	32.1 ± 0.3*
D + PH	3.7 ± 0.3	0.36 ± 0.03	38.1 ± 0.2*	41.6 ± 0.2	31.6 ± 0.4*
A + D + PH	2.8 ± 0.2	0.24 ± 0.02	37.9 ± 0.1*	41.4 ± 0.2	31.6 ± 0.4*

[a] Core temperature, start of run.
[b] Core temperature, end of run.
[c] Tail temperature.
[d] Mean ± S.E.
* Significantly different from controls p < 0.05.

significantly higher than that for all other groups, there was no significant difference among the mean Tc's at EOR.

Tail temperatures (Ti) at SOR (Table II) for the first four groups are just above the 26°C ambient, but it is interesting to note that the Ti SOR of all groups receiving PH were consistently and significantly higher. Fig. 2 illustrates an inverse relationship between the ability to increase tail temperature and the heating rate of the rat. The PH group with the highest heating rate had the smallest increase in Ti, while the A + D group had both the lowest heating rate and the largest increment in Ti.

DISCUSSION

As indicated in Fig. 1, the heating rate of the running rat was a sensitive index of drug effect. We have previously reported that heating rate is a sensitive index of drug activity in a sedentary heat-stressed rat model (16). The lowest heating rates and longest endurance times were in the D and A + D groups, suggesting that D may have a beneficial effect on thermoregulation during exercise. This hypothesis is supported by the work of Vidal et al. (25) who have shown that in the rat hyperthermia induced by handling stress is

reversed by diazepam. Also, successive febrile convulsions in infants can be prevented by diazepam administration (11). The doses of D used by Vidal (25) to obtain a reversal of restraint- or injection-induced hyperthermia were higher than the 500 μg·kg⁻¹ used in the current experiments. We have also observed that a dose of 1.87 mg·kg⁻¹ of D significantly reduced Tc SOR below control levels, thus indicating that the hyperthermia induced by handling may be abolished with D; however, the advantageous effects of D on running performance were achieved by the lower dose (500 μg·kg⁻¹) without effects on Tc SOR or behavior when compared with control groups.

Heat loss through the tail is a major source of heat dissipation in the rat (18,22,23). The lower Ti SOR of the PH, D + PH, and A + D + PH groups, as well as the ability of A to block this lower Tc SOR in the A + PH group, are predicted by the data of Meeter and Wolthuis (18,19). These investigators demonstrated that centrally acting anticholinesterases (such as physostigmine) lower Tc by increasing heat dissipation through the tail. Thus, the lower Ti SOR of all groups receiving PH was consistent with their higher Ti SOR (Table II).
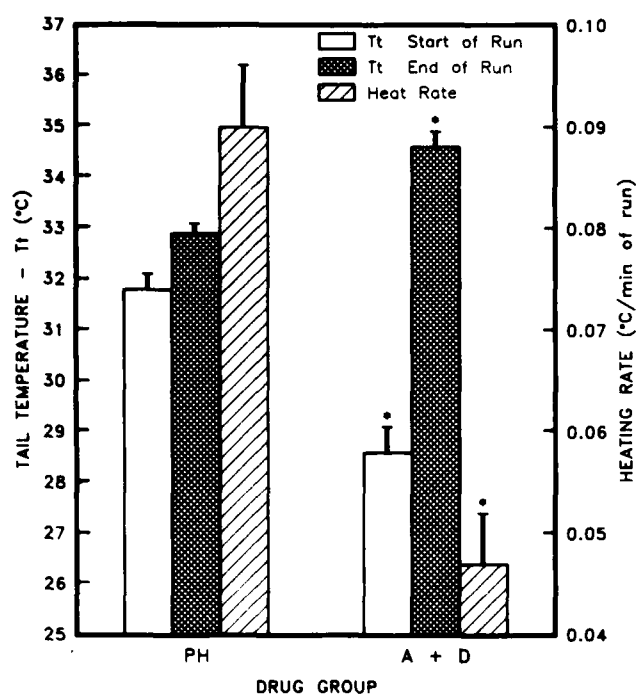
Since a running rat is unable to spread saliva for evapo-

Fig. 2. Tail temperature and heating rates for the PH and A + D groups. Values are mean ± S.E.; * indicates a significant difference (p < 0.05) between the two groups.

rative cooling, heat dissipation through the tail becomes even more important than in a sedentary animal. Voicu et al. (26) demonstrated that the lower T_c of PH-treated rats persists for up to 2 h; however, in exercising rats the blood flow to the working muscles increases at the expense of peripheral blood flow (2). When the rats started exercising, the T_t of PH rats declined and increased only after the rats had become hyperthermic. Fig. 2 illustrates the T_t SOR and EOR for the two groups with the minimal (A + D, 0.047°C·min⁻¹) and maximal (PH, 0.090°C·min⁻¹) heating rates. The A + D group increased its T_t by approximately 6°C from SOR to EOR whereas the PH group only increased its T_t by 1.2°C. T_t SOR for the PH group was higher than that for the A + D group, but during exercise the shift of blood to the working muscles proved to be a thermoregulatory liability for the PH group.

Except for the A group, there were no significant differences among the T_c EOR for any of the groups. The similar T_c at exhaustion may be explained by the observations of Kozlowski et al. (13) whose exercising dogs exhausted after 57 ± 8 min with core temperatures of 41.8 ± 0.2°C, which is very similar to the 53 ± 4 min and 41.6 ± 0.2°C for the C rats. Their data demonstrated that the muscle content of lactate and the temperature of the working muscles were positively correlated and suggested that hyperthermia induced by exercise causes a change in the metabolism of the working muscles which may limit endurance.

The T_t in the A group was the lowest of all the groups at SOR and throughout the observation period. "Atropine flush" (cutaneous vasodilation induced by atropine administration) has been shown by Kolka et al. (12) to be caused in humans by cutaneous vasodilation with increased blood flow, increased skin temperature, and increased conductive

heat loss in the forearm. However, according to O'Leary et al. (21) the neural control of blood flow to the rat tail is analogous to that for apical areas in humans, but not the forearm. Therefore, our observation of a lower T_t in the atropinized rat does not necessarily imply that the rat does not exhibit an "atropine flush," but that it may not occur in the tail.

Administration of the anticholinesterase physostigmine to running rats resulted in reduced endurance and an increased rate of rise in core temperature. The performance decrement and elevated heating rate were both restored to control levels by pretreating the animals with the anticholinergic atropine and the anticonvulsant diazepam. Additionally, diazepam, with or without atropine, seems to improve endurance and thermoregulation in the exercising rat. Further research is required and is being executed to elucidate the mechanism of this improved performance. The combination of A + D + PH appears to be a possible candidate for a prophylactic treatment against organophosphate poisoning, and our running rat model may prove to be a useful tool with which to examine the effects of other anticholinergic and anticholinesterase compounds on physical and thermoregulatory performance.

REFERENCES

1. Aquilonius SM. In: Jenden DJ, ed. Cholinergic mechanisms in the treatment of drug overdose. New York: Plenum Press, 1977: 817–25.
2. Armstrong RB, Laughlin MH. Rat muscle blood flow during high-speed locomotion. J. Appl. Physiol. 1985; 59:1322–8.
3. Craig FN. Inhibition of sweating by salts of hyoscine and hyoscyamine. J. Appl. Physiol. 1970; 28:779–83.
4. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemo. Rpts. 1966; 50:219–44.
5. Goodman LS, Gilman A. The pharmacological basis of therapeutics, 6th ed. New York: MacMillan Co., 1980: 100–36.
6. Hainsworth FR. Saliva spreading activity and body temperature regulation in the rat. Am. J. Physiol. 1967; 212:1288–92.
7. Harris LW, McDonough JH, Stitcher DL, Lennox WJ. Protection against both lethal and behavioral effects of soman. Drug Chem. Toxicol. 1984; 7:605–24.
8. Hubbard RW, Matthew CB, Francesconi RP. Heat-stressed rat: effects of atropine, desalivation, or restraint. J. Appl. Physiol. 1982; 53:1171–4.
9. Hubbard RW, Matthew WT, Criss RFL, Kelly C, Sils I, Mager M, Bowers WD, Wolfe D. Role of physical effort in the etiology of rat heatstroke injury and mortality. J. Appl. Physiol. 1978; 45:463–8.
10. Hubbard RW, Matthew WT, Linduska JD, Curtis FC, Bowers WD, Leav I, Mager M. The laboratory rat as a model for hyperthermic syndromes in humans. Am. J. Physiol. 1976; 231:1119–23.
11. Knudsen FU, Vestermark S. Prophylactic diazepam or phenobarbitone in febrile convulsions: a prospective, controlled study. Arch. Dis. Child. 1978; 53:660–3.

12. Kolka MA, Stephenson LA, Bruttig SP, Gonzalez RR. Cutaneous blood flow and local sweating during exercise after atropine [Abstract]. Fed. Proc. 1986; 45:407.

13. Kozlowski S, Brzezinska Z, Kruk B, Kaciuba-Uscilko H, Greenleaf JE, Nazar K. Exercise hyperthermia as a factor limiting physical performance: temperature effect on muscle metabolism. J. Appl. Physiol. 1985; 59:766–73.

14. Larson GF, Hurlbert BJ, Wingard DW. Physostigmine reversal of diazepam-induced depression. Anesth. Analg. 1977; 56:348–51.

15. Matthew CB, Hubbard RW, Francesconi RP. A heat-stressed rat model to determine relative anticholinergic and anticholinesterase drug potency. Aviat. Space Environ. Med. 1986; 57:1061–5.

16. Matthew CB, Hubbard RW, Francesconi RP, Szlyk PC. An atropinized heat-stressed rat model: dose response effects and pharmacokinetics. Aviat. Space Environ. Med. 1986; 57:659–63.

17. Matthew WT, Szlyk PC. A microcomputer controlled temperature data acquisition system employing rapid, high precision calibration of thermistors or platinum RTD's [Abstract]. Physiologist 1984; 27:248.

18. Meeter E. The mode of action of cholinesterase inhibitors on the temperature regulation of the rat. Arch. Int. Pharmacodyn. 1969; 182:416–9.

19. Meeter E, Wolthuis OL. The effects of cholinesterase inhibitors on the body temperature of the rat. Eur. J. Pharmacol. 1968; 4:18–24.

20. Nagy J, Decsi L. Physostigmine, a highly potent antidote for acute experimental diazepam intoxication. Neuropharmacology 1978; 17:469–75.

21. O'Leary DS, Johnson JM, Taylor WF. Mode of neural control mediating rat tail vasodilation during heating. J. Appl. Physiol. 1985; 59:1533–8.

22. Raman ER, Roberts MF, Vanhuyse VJ. Body temperature control of rat tail blood flow. Am. J. Physiol. 1983; 14:R426–32.

23. Rand RP, Burton AC, Ing T. The tail of the rat in temperature regulation and acclimatization. Can. J. Physiol. Pharmacol. 1965; 43:257–67.

24. Rump S, Grudzinska E. Investigations on the effects of diazepam in acute experimental intoxication with fluostigmine. Arch. Toxikol. 1974; 31:223–32.

25. Vidal C, Suaudeau C, Jacob J. Hyper- and hypothermia induced by nonnoxious stress: effects of naloxone, diazepam and gamma-acetylenic GABA. Life Sci. 1983; 33 (Suppl 1):587–90.

26. Voicu V, Jiquidi M, Sitcai N, Bruja NA. Some pharmacological correlations of hypothermia induced by anticholinesterasics. Physiologie 1976; 13:285–91.